Metabolome analysis of urine

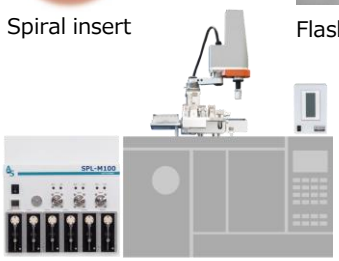
by online solid-phase analytical derivatization GC-MS system

Introduction

Solid-phase derivatization (SPD) is a technique of derivatization without the time-consuming centrifugal concentration and lyophylization, by retaining the target compounds on a solid phase and dewatering it by passing a organic solvent through it then infiltrating the derivatization reagent and performing the reaction on the solid phase. The example of pretreatment method and analytical condition for metabolome analysis of urine are shown below.

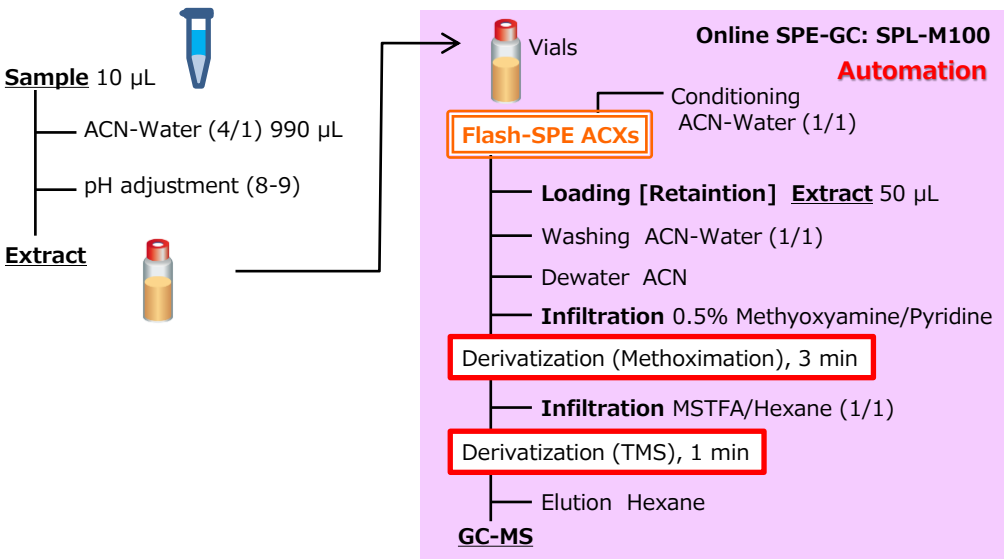
Spiral insert

Flash-SPE



Online SPE-GC-MS system

SPD pretreatment workflow



Analytical condition

SPE-GC interface	**SPL-M100 (AiSTI SCIENCE)**
SPE cartridge	Flash-SPE
PTV injection port	**LVI-S250 (AiSTI SCIENCE)**
Insert type	Spiral insert
Temp.	220℃(0.5 min)-50℃/min-290℃(23 min)
Gas chromatograph	
Inlet mode	Split 1:50
Flow mode	Constant flow, 1.0 mL/min
Pre-column	0.25 mm i.d. x 0.5 m
Column	Vf-5ms, 0.25 mm i.d. x 30 m, df=0.25 µm
Oven Temp.	100℃(2 min)-10℃/min-320℃(2min)
Transfer line Temp.	290℃
Mass spectrometer	
Acquisition mode	Scan (*m/z* 70-600)
Data acquisition	3.0-26 min

SPL-M100
for SPE-GC system

Sample

Information

AiSTI SCIENCE

Product

Online SPE-GC
SPL-M100
Solid-phase cartridge
Flash-SPE
GC large volume injection port
LVI-S250

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Results

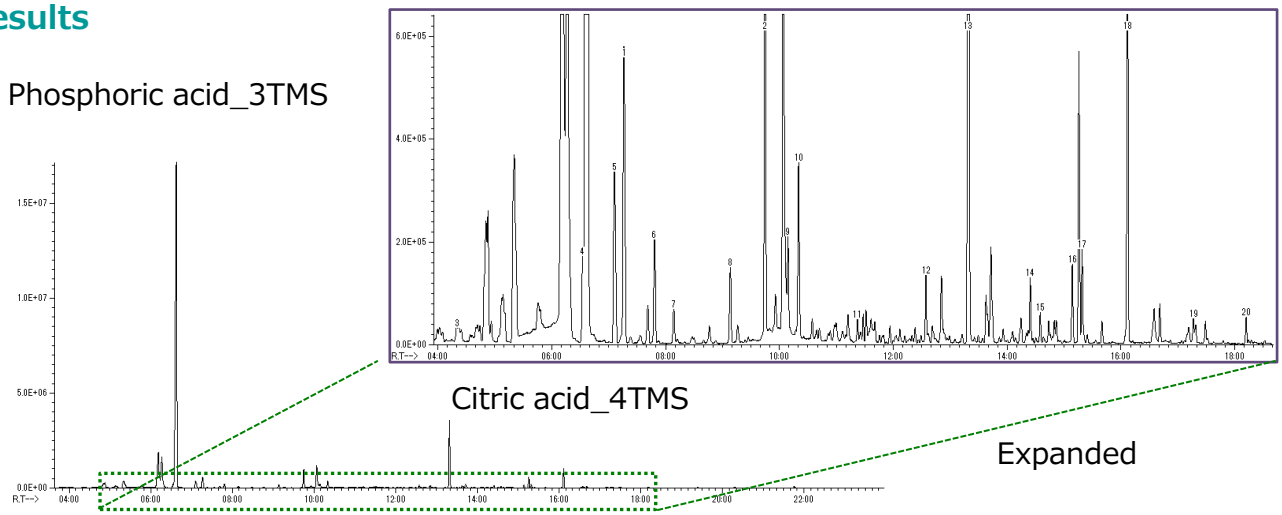

Figure: Total ion current chromatogram

Table 1: Result of recovery test (n=5)

Sample	No.	Norleucine_2TMS	Adipic acid_2TMS
Standard solution	S1	1,284,451	84,108
IS concentrations:	S2	1,243,248	77,955
20 μM in vials	S3	1,169,708	73,754
	S4	1,183,814	73,368
	S5	1,131,803	73,641
	Ave.	*1,202,605*	*76,565*
	RSD, %	***5.1***	***6.0***

Sample	No.	Norleucine_2TMS	Adipic acid_2TMS
Urine	Feces_K1	1,400,444	99,683
Dilution:	Feces_K2	1,415,794	98,689
100 times	Feces_K3	1,395,904	99,474
Spike period of IS:	Feces_K4	1,415,822	98,843
After deprotenization,	Feces_K5	1,391,498	96,746
20 μM in vials	*Ave.*	*1,403,892*	*98,687*
	RSD, %	***0.8***	***1.2***
(K/Sx100)	Recovery, %	**117**	**129**
Mouse Feces	Feces_A1	1,451,203	106,679
Dilution:	Feces_A2	1,521,654	103,472
100 times	Feces_A3	1,477,161	99,614
Spike period of IS:	Feces_A4	1,434,121	100,407
Before extraction	Feces_A5	1,533,918	98,048
2 mM in urine (20 μM in vials)	*Ave.*	*1,483,611*	*101,644*
	RSD, %	***2.9***	***3.4***
(A/Sx100)	Recovery, %	**123**	**133**
(A/Kx100)	Recovery, %	**106**	**103**

Table 2: Result of repeatability test (RSD%, n=5)

No.	Metabolites	1	2	3	4	5	*Ave.*	**RSD, %**
1	Alanine_2TMS	180,899	188,241	182,938	187,713	183,349	*184,628*	**1.7**
2	Ethanolamine_3TMS	486,231	499,319	511,776	491,099	499,867	*497,658*	**2.0**
3	Glycine_3TMS	113,691	111,586	113,681	113,461	115,306	*113,545*	**1.2**
4	Serine_3TMS	137,104	142,004	135,803	142,205	140,908	*139,605*	**2.1**
5	Threonine_3TMS	12,057	13,193	12,294	12,865	12,957	*12,673*	**3.8**
6	3-Aminoisobutyric acid_3	35,108	34,875	34,061	39,025	35,149	*35,644*	**5.4**
7	Threonic acid_4TMS	46,558	43,938	43,553	46,164	45,096	*45,062*	**2.9**
8	Creatine_3TMS	71,004	68,064	69,480	69,156	73,464	*70,234*	**3.0**
9	p-Hydroxyphenylactic aci	7,510	7,593	7,418	7,995	8,074	*7,718*	**3.8**
10	Aconitic acid_3TMS	18,060	17,740	18,623	18,335	17,708	*18,093*	**2.2**
11	Citric acid_4TMS	319,248	313,843	321,899	328,974	324,604	*321,714*	**1.8**
12	L-Lysine_4TMS	26,531	26,314	27,691	25,908	27,728	*26,834*	**3.1**
13	Tyrosine_3TMS	67,041	67,289	65,806	67,529	66,123	*66,758*	**1.1**
14	Gluconic acid_6TMS	23,901	21,094	22,881	23,351	20,520	*22,349*	**6.6**
15	Glucopyranuronic acid_5	90,468	97,764	91,691	99,144	93,550	*94,523*	**4.0**
16	Uric acid-4TMS	358,265	365,984	362,302	356,388	382,576	*365,103*	**2.9**